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Preliminary phytochemical screening and Invitro antioxidant activities of *"Antidesma alexitera L."* leaf extract

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ABSTRACT

There is no plant in the world that is non- medicinal, or which cannot be used as medicine is used as medicine. Folk or traditional systems of medicines always played an imperative role in the global healthcare system. Antidesma alexiteria.L (Thalimaram), belongs to the Phyllanthaceae family. Antidesma species has been used in many traditional medicines to treat diarrhea, skin complaints, hemorrhages, and abdominal disorders. The present study focuses on the preliminary Phytochemical screening and evaluation of the antioxidant potential of Antidesma alexiteria.L .For qualitative analysis ,standard tests were used to identify phytochemicals present in the extract and confirmed the presence of carbohydrates, coumarins, glycosides, saponins, phenols and phytosterols. Leaf extracts were also subjected for total phenolic content(TPC), total tannin content (TTC) and total flavonoid content(TFC). In quantitative estimation, TPC content of each extract was 24.13, 32.65 & 3.89 mg of GA/g extract, TFC was 230.38, 493.85 &217.15 mg of Ru/g extract and total tannin were 613, 503 &39.67 mg of CTN/g extract respectively. Antioxidant properties of the three extracts were evaluated using DPPH assay, Nitric oxide scavenging activity and total antioxidant activity. Ascorbic acid was used as standard. In DPPH free radical scavenging assay IC50 value of alcoholic, hydroalcoholic and aqueous root extracts was found to be 650.7,534.1 and 7704.18µg/mL respectively. For Nitric Oxide Radical Scavenging assay IC50 value of alcoholic, hydroalcoholic and aqueous root extracts was found to be 928.9, 354.6 and 380.24µg/mL respectively. The Total antioxidant activity of the plant extracts was calculated from the calibration curve of ascorbic acid, and the results showed 49.76, 44.97 and84.14 mg of AA/g activity for ethanolic, hydroethanolic and aqueous extracts respectively

Keywords— *Antidesma alexiteria. L*, antioxidant potentials, leaf extracts, phytochemical constituents

1. INTRODUCTION

Traditional folk/herbal medicinal practitioners have described the therapeutic benefits of many indigenous plant species, the natural plant extracts are the source of herbal medicine. *Antidesma alexitera. L* is an example for such plants.

Antidesma alexitera.L belongs to the Phyllanthaceae family; it was previously classified under Euphorbiaceae family. This species is indigenous to South India and Sri Lanka commonly known as " thalimaram" in Kerala and "Heen embilla/Hinembilla" in Sri Lanka. *Antidesma alexiteria.L is* dioecious trees or shrubs with a simple indumentum and its fruits drupaceous, edible, and red to black. [9]

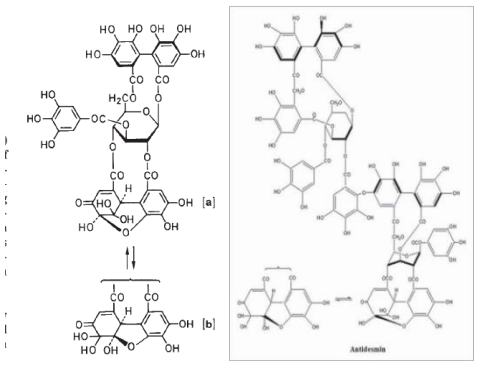
A.alexiteria.L fruit has potential antioxidants which stabilizes oxidation in edible oils[2] and can be used as natural food colour[1]. Its leaves are used as an antidote for snake bites: by the tribal in Tirunelveli Hills, Western Ghats.[7] and young leaves are boiled

with potherbs in case of syphilis in India[3]. The bark fibre is used to make cord and root bark is said to be a cure for dysentery [4]. Based on traditional knowledge local communities use its leaves as herbal shampoo.

(a) Fruit **(b) Inflorescence** **(c) leaf upper side** **(d) Petiole and leaf Insertion**

Fig 1: *Antidesma alexiteria.L* (leaf, fruit & inflorescence)

In Southeast Asian folk medicine different Antidesma species were used to treat diarrhoea, skin complaints, haemorrhages, and abdominal disorders, for which tannin components could possibly be an active principle. Two important hydrolysable tannins "carpusin" and a novel dimer "antidesmin A" [8,5] were reported from Euphorbiaceae plants. The anti-cholesteric activity of *Antidesma pentandruin Merr*an is due to the presence of triterpene (lupeolactone) [8] .For the first time *Antidesma alexitera.L.*" was selected to evaluate its antioxidant potential and screening of phytochemicals present in it.



(i) **(ii)**

Fig 2: Tannin (i) novel dimer "antidesmin" structure (ii) carpusin structure [8,5]

2. MATERIAL AND METHODS

2.1 Plant Material

Leaves of *A.alexiteria.L* were collected from Trivandrum district of Kerala, India. A voucher specimen (38314,38315) has been deposited at the herbarium of the Institute. The leaves were washed, and shade dried then ground to coarse powder with a mechanical grinder. Preparation of Plant Extract: 100g of the leaf powder was cold extracted (using magnetic stirrer) with 100% ethanol for 3hrs. The extract was filtered using whattman no.1 filter paper and the residue was replenished with fresh solvent for next round of extraction. The extraction step was totally carried out for 3 times to maximize the extract yield. The collected filtrates were pooled together and concentrated in a rotary vacuum evaporator at 40℃ to obtain ethanolic extract. The extraction was repeated sequentially with 50% ethanol in water and 100% water and the collected filtrates were concentrated in a rotary vacuum evaporator at 40oC to obtain 50% hydroalcoholic and aqueous extracts respectively. The condensed extract was used for preliminary phytochemical screening and to test its antioxidant activities.

2.2 Preliminary Phytochemical analysis

Comparative preliminary phytochemical study on crude and various fractions of *A.alexiteria.L* were carried out using standard procedures [14,16,15] in order to detect the presence of phytoconstituents such as alkaloids, phenols, flavonoids, Phyto steroids, saponins, glycosides and carbohydrates.(Table 1)

2.3 Quantitative phytochemical analysis

(a) ***Estimation of Total phenolic content (TPC):*** This was estimated by spectrophotometry according to Singleton and Rossi, 1965 [17], method. 0.5 ml of extract was transferred into tubes containing 2.5ml 10% Folin-Ciocalteu's reagent. After 10 min, 2ml of sodium carbonate solution was added to the sample and it was allowed to stand at room temperature for 30 min. Absorbance was read at 743nm, the concentration of polyphenols in the sample was derived from a standard curve of Gallic acid. The TPC was expressed as Gallic acid equivalents (GAE) in mg/g of dry extract.

(b) ***Estimation of Total Flavonoids (TFC):*** it is determined according to the Aluminium chloride colorimetric method of Chang *et al*,2003 [18] with slight modification. Plant extract (1mg/ml) in methanol was mixed with 0.1 mL of 10% Aluminium chloride hexahydrate, 0.1ml of 1M potassium acetate and 2.8ml of deionized water. After 30 min incubation at room temperature, the absorbance of the reaction mixture was determined spectrophotometricaly at 415nm. Rutin was taken as a standard (con range

12.5 to 400µg/ml), the total flavonoid content was calculated from the standard curve and expressed as Rutin equivalents in mg/g of dry extracts

(c) ***Total condensed Tannin****:* Condensed tannins were colorimetrically estimated by vanillin hydrochloride method [19]. The condensed tannins content was calculated from the calibration curve of standard catechin ranging between 20-100µg/ml. Tubes containing 1ml each of standard/ plant extract in methanol were incubated at 30 °C in a water bath and this 5ml of working reagent was added to an interval of 1 min to one set of the tubes and 5ml of 4% HCL was added to the other set at intervals of 1.0 min. Kept the samples in the water bath for 20min and the absorbance was read at 500nm. The absorbance of the blank was subtracted from that of the sample containing vanillin reagent.

2.4 Antioxidant assay

(a) ***Nitric oxide scavenging assay [13]:***Sodium nitroprusside (10mM) was mixed with 1ml of different plant extract concentration in phosphate buffer pH 7.4. The mixture was incubated at 25 °C for 150 min. To 500µl of the incubated solution, 500µl of Griess reagent was added, absorbance was measured at 546nm and percentage of incubation was calculated. Ascorbic acid was used as a standard.

(b) ***DPPH Radical Scavenging assay [11]:*** Methanolic solution of 2ml DPPH in 10ml was added to different con of plant extracts & allowed to react at room temperature for 30 min in the dark. Absorbance was taken at 517nm, methanol was used as blank and 200µl of methanol was added to DPPH in positive control tube. Ascorbic acid was used as standard. The percentage of radical scavenging of samples was calculated.

(c) ***Total Antioxidant activity:*** This was analysed by phosphomolybdenum method [12]. 200µl of extract in respective solvent was mixed with 2ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate & 4mM ammonium molybdate). The reaction mixture was incubated at 95 °C for 90 min. Absorbance of the sample was measured at 635nmm. The total antioxidant capacity of the plant was calculated from the calibration curve of ascorbic acid and expressed as µg ascorbic acid equivalents /g of dry extracts

Statistical Analysis: The results obtained were expressed as mean(+/-) standard deviation and presented as graphs and tables.

3. RESULTS AND DISCUSSION

3.1 Preliminary phytochemical analysis

Table 1: Qualitative Phytochemical analysis results

No	Phytochemicals	Test	Ethanolic extract(AAE)	Hydroethanolic extract(AAH)	Aqueous extract(AAA)
1	ALKALOIDS	Wagner's test	-	-	-
		Hager's test	-	-	-
		Dragendroff's test	-	-	-
2	FLAVONOIDS	Shinoda's test	-	-	+
3	CARBOHYDRATE	Fehling's test	+ (reddish orange ppt)	+ (reddish orange ppt)	+ (reddish orange ppt)
		Benedict test	+ (red ppt)	+ (red ppt)	-
4	COUMARINS		+	+	+
5	GLYCOSIDES	Bontrager's test	-	-	-
		Legal's test	+	+	-
		Keller Kiliani test	+	+	+
6	SAPONINS	Foam test	-	+	+
7	PHYTOSTEROL & TERPENOIDS	Salkowski test	-	+ **(golden yellow- terpenes)**	+ **(red- sterols)**
8	PROTEINS & AMINO ACIDS	Millon's Test	-	-	-
		Biuret's test	-	-	-
		Ninhydrin test	-	-	-
9	PHENOLIC COMPOUNDS & TANNIN	Ferric Chloride test	+	+	+
		Lead Acetate test	-	+	+
10	GUM & MUCILAGE		-	-	-
11	FIXED OILS & FATS	Spot test	-	-	-
		Saponification test	-	-	-

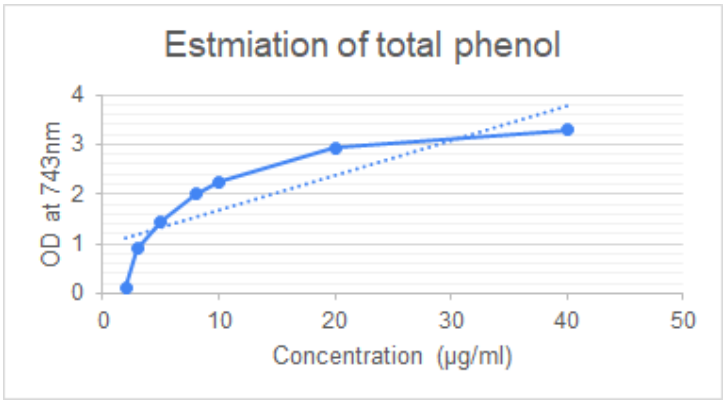
+present – absent

The preliminary phytochemical analysis of ethanolic ,hydroethanolic ,and aqueous extracts of *Antidesma alexitera* leaves shows the presence or absence of various phytochemical constituents. The presence of phenolic compounds, carbohydrates, glycosides and coumarin was found in ethanolic, hydroethanolic and aqueous extract. Saponins, phytosterols and terpenoids were found in

hydroethanolic and aqueous extract. Only in aqueous extract showed positive results for flavonoids. So, when compared to ethanolic extract hydroethanolic and aqueous extract have more phytochemical compounds.

3.2 Quantitative Phytochemical Analysis

Determination of total phenolic content

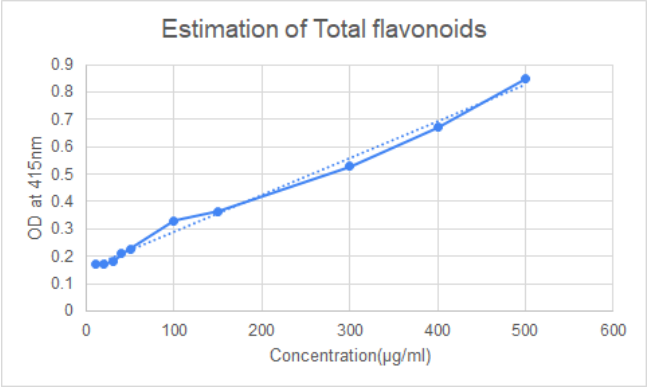


Graph 1: Total phenol estimation, OD at 743nm against the concentration.

Table 2: The total phenolic content calculated from Graph 1

Concentration of test	Total phenolic content(µg/ml)
Ethanolic extract- AAE (0.5ml)	24.13
Hydroethanolic extract- AAH (0.5ml)	32.65
Aqueous extract- AAA (0.5ml)	3.89

Estimation of total flavonoids

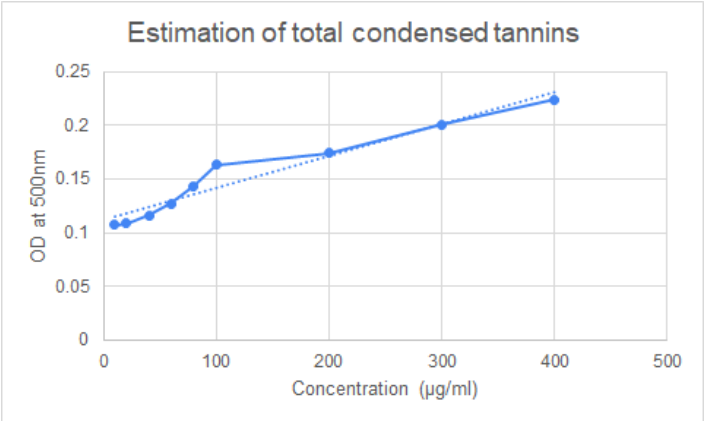


Graph 2: Total flavonoid estimation, OD at 415nm against the concentration

Table 3: The total flavonoids content calculated from Graph 2

Concentration of test	Total flavonoids (µg/ml)
Ethanolic extract- AAE (0.5ml)	230.38
Hydroethanolic extract- AAH (0.5ml)	493.85
Aqueous extract- AAA (0.5ml)	217.15

Estimation of Total Condensed Tannins



Graph 3: Total condensed tannin, OD at 500nm against the concentration.

Table 4: The total condensed tannin calculated from Graph 3

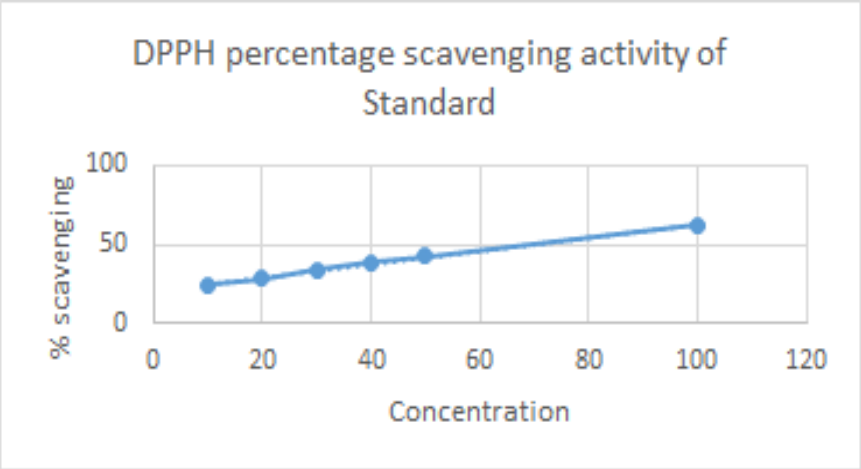
Concentration of test	Total Condensed Tannins (µg/ml)
Ethanolic extract- AAE (0.5ml)	613
Hydroethanolic extract-AAH (0.5ml)	503
Aqueous extract-AAA (0.5ml)	39.67

3.3 Antioxidant Assay

DPPH radical scavenging activity

Table 5: DPPH radical scavenging assay,

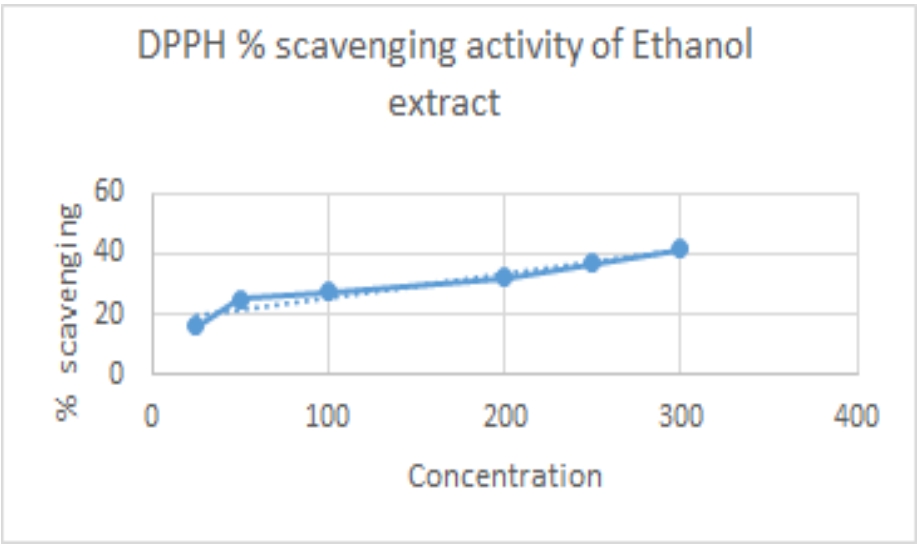
Concentration of standard (µg/ml)(Ascorbic acid)	%scavenging activity	IC50
20	24.84	391.60
40	38.63	
60	43.78	
80	50.20	
100	62.15	

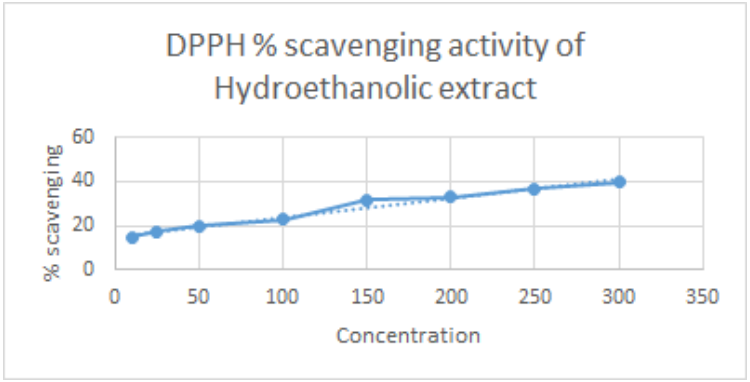


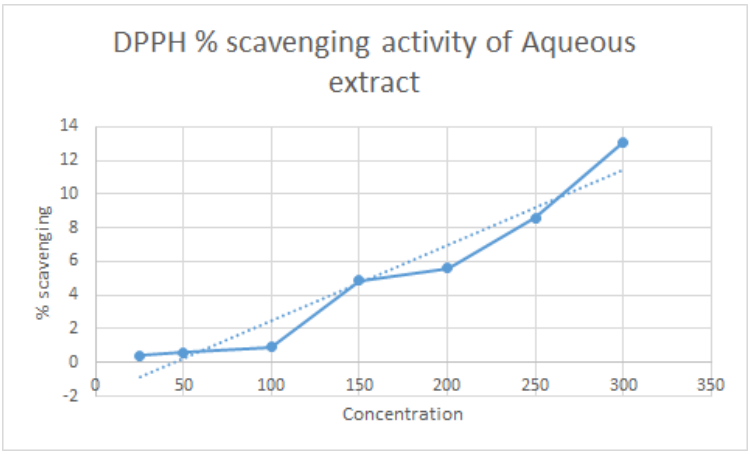
Graph 4: DPPH scavenging activity of standard

Table 6: Percentage scavenging activity of ethanolic hydroethanolic and aqueous extract at various concentrations

Concentration of extracts(µg/ml)	Ethanolic extract percentage scavenging activity - AAE	Hydroethanolic extract Percentage scavenging activity- AAH	Aqueous extract percentage scavenging activity- AAA
25	16.29	14.87	0.40
50	25.13	17.28	0.61
100	27.20	19.81	0.94
150	31.29	22.93	4.86
200	31.56	31.51	5.61
250	38.85	36.83	8.60
300	41.69	39.87	13.09







Graph 5: DPPH scavenging activity of ethanolic, hydroethanolic and aqueous extract

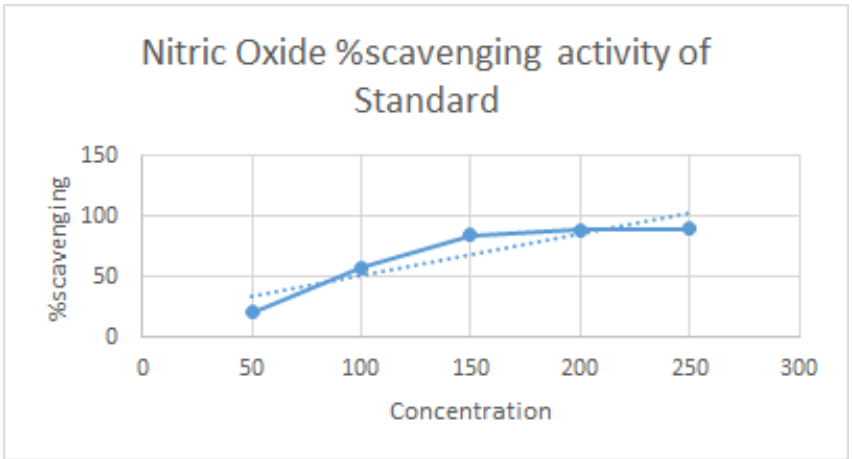
Table 7: IC_{50} value of ethanolic, hydroethanolic, aqueous and standard as follows

Sample/Standard	IC_{50}
Ethanolic extract- AAE	650.65
Hydroethanolic extract-AAH	534.05
Aqueous extract- AAA	7704.18
Standard	391.60

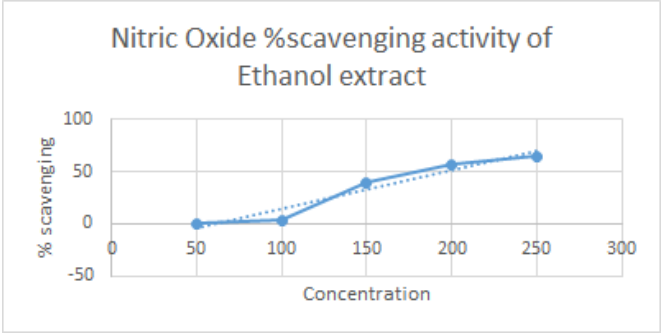
Nitric Oxide radical scavenging activity

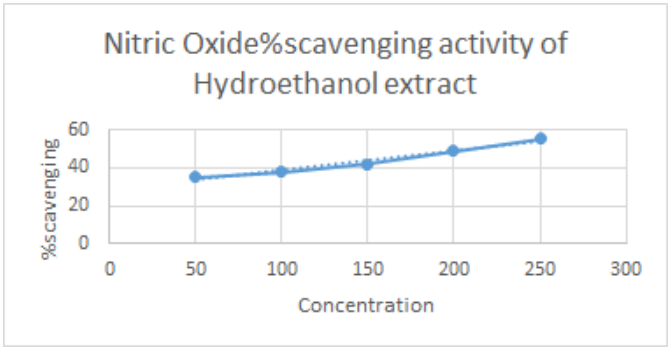
Table 8: Nitric oxide radical scavenging activity of ascorbic acid (standard) and % scavenging activity

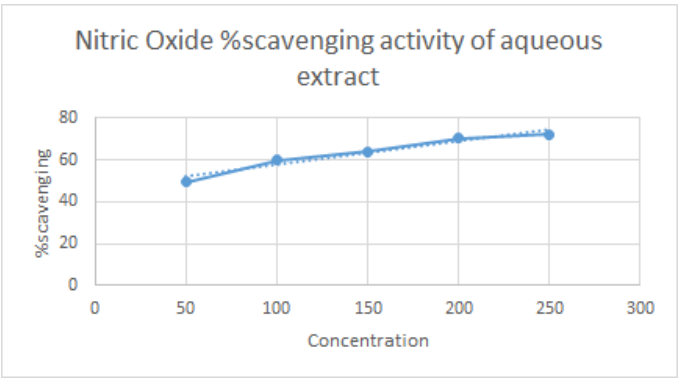
Concentration of standard (µg/ml) (Ascorbic acid)	Percentage scavenging activity	IC_{50} of Standard
50	20.63	82.21
100	58.08	
150	84.63	
200	89.14	
250	19.73	



Graph 6: nitric oxide percentage scavenging activity of standard ascorbic acid





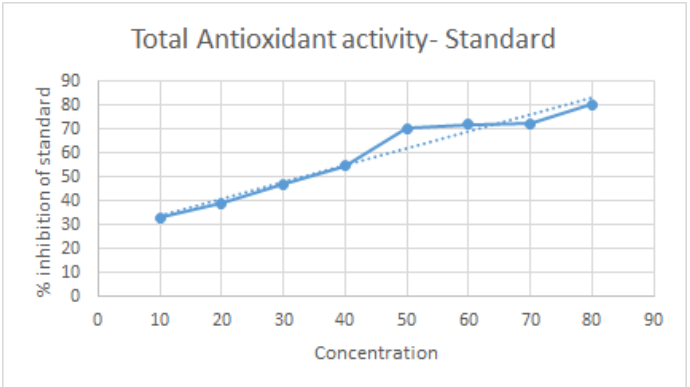


Graph 7: nitric oxide percentage scavenging activity of ethanolic, hydroethanolic and aqueous extract

Table 9: Nitric oxide percentage scavenging activity of ethanolic hydroethanolic and aqueous extract at various concentrations along with its IC_{50} value is as follows

Concentration of extracts (µg/ml)	**Ethanolic extract percentage scavenging activity- AAE**	**IC_{50}** of Ethanolic extract	**Hydroethanolic extract Percentage scavenging activity- AAH**	**IC_{50}** of Hydroethanolic extract	**Aqueous extract percentage scavenging activity- AAA**	**IC_{50}** of Aqueous extract
50	0.12134	928.95	35.2996	354.64	49.3396	380.24
100	2.95875		37.7123		59.8539	
150	39.4437		41.8471		64.047	
200	56.5382		48.698		70.1162	
250	64.4531		55.2828		72.2312	

Total antioxidant activity



Graph 8: the total antioxidant activity of standard

Determination of Total antioxidant activity

Table 10: total antioxidant activity of the standard

Concentration of standard (µg/ml) (Ascorbic acid)	Mean absorbance (635 nm)
10	0.220
20	0.2922
30	0.3745
40	0.4234
50	0.8860
Control	0.0823

Table 10: total antioxidant activity of three extracts

Concentration of test (µg/ml)	Total antioxidant activity
Ethanolic extract- AAE (200µg/ml)	49.76
Hydroethanolic extract- AAH(200µg/ml)	44.98
Aqueous extract- AAA (200µg/ml)	84.14

The results of the study revealed that hydroethanolic and aqueous extract possess more phytochemical compounds than ethanolic extract. In quantitative estimation, total phenolic content of each extract was 24.13, 32.65 & 3.89 mg of GA/g extract, total flavonoid was 230.38, 493.85 &217.15 mg of Ru/g extract and total tannin were 613, 503 &39.67 mg of CTN/g extract respectively. Natural extracts with proven antioxidant activity are typically composed with their phenolic moiety, for instance flavonoids, coumarins and tocopherols and these are present in all the extracts. Organic acids, carotenoids and tannins may also be present and act as antioxidants or have a synergistic impact with phenolic compounds

The DPPH assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple colour. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. .In DPPH free radical scavenging assay IC50 value of alcoholic, hydroalcoholic and aqueous root extracts was found to be 650.7, 534.1, 7704.18µg/mL respectively.

For Nitric Oxide Radical Scavenging assay IC50 value of alcoholic, hydroalcoholic and aqueous root extracts was found to be 928.95, 354.64, 380.24 µg/mL respectively. The total antioxidant activity of the plant extracts was calculated from the calibration curve of ascorbic acid ,the results showed the antioxidant activity of ethanolic, hydroethanolic and aqueous extracts were 49.76, 44.97 and 84.14 mg of AA/g extract respectively.

This study was able to prove the antioxidant potential of *Antidesma alexitera* leaves, further studies must be done to find out its medicinal potential. These extracts should undergo purification and isolation process to find out its phytochemicals because for all the assay and tests crude ethanolic, hydroethanolic and aqueous extracts were used. Due to human error percentage scavenging assays results vary by small digits.

4. CONCLUSION

Based on the results obtained in the present study, it is concluded that hydroethanolic extract of *Antidesma alexitera* leaf, possess large amounts of flavonoid, phenolics and tannin content. In qualitative analysis, the phytochemical compounds such as carbohydrates, coumarins, glycosides, saponins, phenols and phytosterols were screened using standard methods, and the results showed the hydroethanolic and aqueous extract possess more phytochemical constituents when compared with ethanolic extract. In quantitative estimation, total phenolic content of each extract 24.13, 32.65 & 3.89 mg of GA/g extract, total flavonoid was 230.38, 493.85 &217.15 mg of Ru/g extract and total tannins were 613, 503 &39.67 mg of CTN/g extract respectively. So hydroethanolic extract of *Antidesma alexitera* leaf has a high amount of flavonoid, phenolic and tannin content, when compared with other extracts. The presence of the identified phytochemical components makes the leaves pharmacologically active, which exhibits high antioxidant and free radical scavenging activities. Antioxidant assays such as, DPPH radical scavenging activity and total antioxidant activities of ethanolic hydroethanolic and aqueous extracts of *Antidesma alexiteria.L* were compared with the standard ascorbic acid curve. Hydroethanolic extract of *Antidesma alexiteria.L* leaves has got profound antioxidant potential when compared with the ethanolic and aqueous extracts. *Antidesma alexiteria.L* is a good candidate as it is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stress related diseases. Thus, the present study concluded that, natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug leads because of their unmatched availability of chemical diversity and further studies on *Antidesma alexiteria.L* is warranted.

5. ACKNOWLEDGEMENT

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Author's contribution

NG and MTS performed the experimental work as per the guidance of SRS and BBS in designing the protocol. NG and MTS completed the report and project paper and SRS ,and BBS and RPK reviewed the manuscript.

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